


We claim:

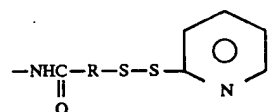
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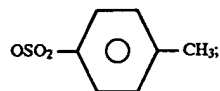
$\text{—OSO}_2\text{—}$  —CH_3 —NH_2

$\text{—NHC(=O)—R—N(=C(=O)CH}_2\text{CH}_2\text{C(=O)—}$

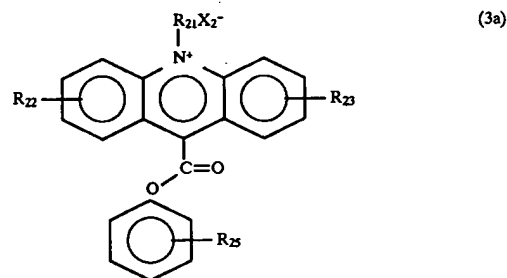
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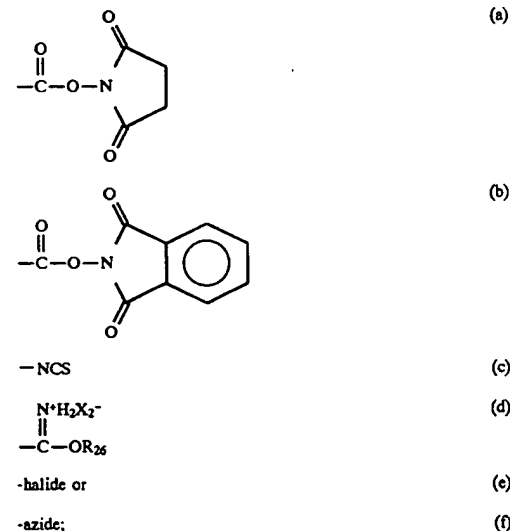
X_1 is $CH_3SO_4^-$, OSO_2F^- , a halide, $OSO_2CF_3^-$, $OSO_2C_4F_9^-$, or



and R is an alkyl, aryl or aralkyl group; or said ester is a compound of the formula (3a):



wherein R₂₅ comprises one of the following:



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R_{21} and R_{26} are independently a hydrogen atom or C_1-C_{10} optionally substituted alkyl, alkenyl, alkynyl or aryl group; R_{22} and R_{23} are independently a hydrogen atom, halide atom, amino, substituted amino, carboxyl, hydroxyl, alkoxy or nitro group; and X_2 is a halide atom, comprising reacting at a pH of 6 to 10 said acridinium ester and a superoxide anion produced in situ by electrochemical reduction of oxygen dissolved in an electrolyte using electrodes in the presence of a flavin compound, wherein said acridinium ester is attached as a label to a reagent in said immunoassay, hybridization assay or immunoblot assay.

2. The method of claim 1, wherein the electrochemical reduction is performed in the presence of $1 \times 10^{-9} M$ to $1 \times 10^{-4} M$ of the flavin compound at a potential of -0.3 to $-0.7 V$ (vs. $Ag/AgCl$).

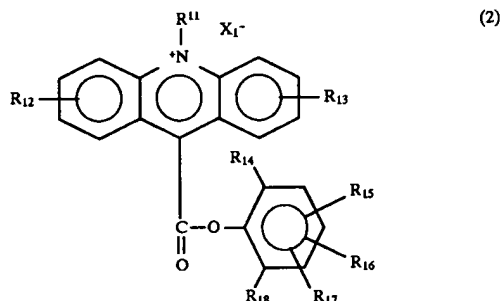
3. The method of claim 1 wherein the electrochemical reduction comprises use of an electrode comprising said flavin compound.

4. The method of claim 1, wherein the acridinium ester is 4-[2-succinimyl(oxycarbonyl)ethyl]phenyl-10-methylacridinium-9-carboxylate fluorosulfate.

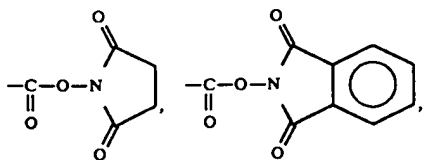
5. A method of detecting a substance to be examined in a sample in an immunoassay, hybridization assay or immunoblot assay, comprising,

binding a chemiluminescent labeled substance having affinity for said substance to be examined with said substance to be examined in a sample to produce a complex of said substance to be examined and said chemiluminescent labelled substance having affinity therefor; separating said complex;

reacting said chemiluminescent label with a superoxide anion at a pH of 6 to 10, said label being an acridinium ester of the formula (2):

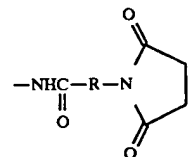
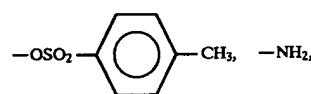
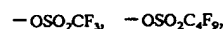
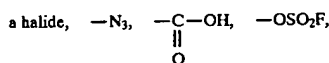
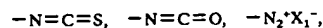
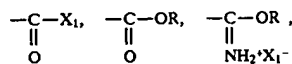
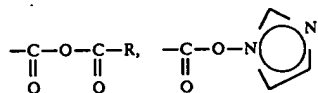


wherein R_{11} is an alkyl, alkenyl, alkynyl or aryl group; R_{12} , R_{13} , R_{15} and R_{17} are independently a hydrogen atom, halide atom, amino, carboxyl, hydroxyl, alkoxy or nitro group; R_{14} and R_{18} are independently an alkyl, alkenyl, alkynyl, aryl, alkoxy, amino, amido, sulfonamido or sulfide group; R_{16} is $-R_{19}-R_{20}$; R_{19} is not required but optionally can be an alkyl, aryl or aralkyl group; R_{20} is selected from the group consisting of:

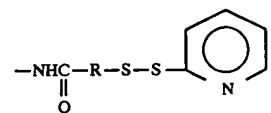


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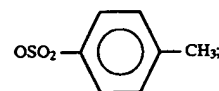
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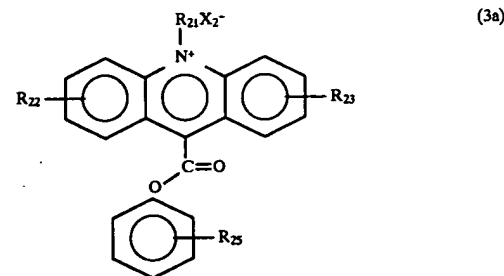
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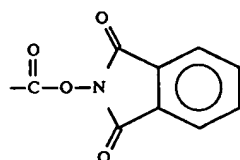
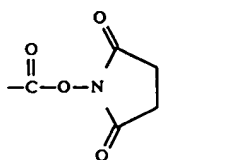
X_1 is CH_3SO_4- , OSO_2F- , a halide, OSO_2CF_3- , $OSO_2C_4F_9-$, or



and R is an alkyl, aryl or aralkyl group; or said label is an acridinium ester of the formula (3a):



wherein R_{25} comprises one of the following:



-NCS



-continued

- (a) -halide or (e)
-azide; (f)
- 5 R_{21} and R_{26} are independently a hydrogen atom or C_1-C_{10} optionally substituted alkyl, alkenyl, alkynyl or aryl group; R_{22} and R_{23} are independently a hydrogen atom, halide atom, amino, substituted amino, carboxyl, hydroxyl, alkoxyl or nitro group; and X_2 is a halide atom; wherein said superoxide anion is produced in situ by electrochemical reduction of oxygen dissolved in an electrolyte using electrodes in the presence of a flavin compound; and
- (b) 10 measuring luminescence of said chemiluminescent label to detect said substance to be examined.
- 15 6. The method of claim 5 wherein said binding step in said hybridization assay is reaction between a nucleic acid and a nucleic acid complementary thereto.
- (c) 7. The method of claim 5, wherein the acridinium ester is
- (d) 20 4-[2-(succinimzyloxy carbonyl)ethyl]phenyl-10-methylacridinium-9-carboxylate fluorosulfate.]

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add
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